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PRINCIPAL INVESTIGATOR: Robert E. Reiter, M.D.

CONTRACTING ORGANIZATION: The University of California,
Los Angeles
Los Angeles, CA 90024-1406

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Robert E. Reiter, M.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

The University of California, Los Angeles
Los Angeles, CA 90024-1406

E-Mail: rreiter@mednet.ucla.edu

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13. ABSTRACT (Maximum 200 Words)

PSCA is a cell surface protein identified in our laboratory, which is expressed in a majority of prostate cancers. The major goals of this grant were to determine the expression of PSCA in localized and metastatic prostate cancer, to determine if expression correlated with clinicopathologic features of prostate cancer, and whether PSCA expression could be detected in bone marrow. Over the past year, we have completed the major portions of Aim 1, determining PSCA expression in a prostate cancer tissue microarray and in a large number of prostate cancer metastases. This has resulted in a publication in press at the Journal of Urology. The major finding is that PSCA expression increases with tumor stage and grade and correlates with a higher risk of cancer recurrence. PSCA is also expressed in 85% of bone metastases, making it a reasonable target for antibody therapy. A prospective study of PSCA in biopsies is underway.

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INTRODUCTION

PSCA is a cell surface antigen expressed in normal prostate, which was previously shown by our group to be overexpressed (when compared to expression in normal prostate) in a significant percentage (30-40%) of prostate cancers. Our initial analysis suggested that PSCA expression might be higher in tumors of higher Gleason score and stage and in bone metastases. The major goal of the current proposal was to establish prostate tissue arrays and test the hypothesis that PSCA expression could be used prognostically. We also wanted to see if PSCA could identify patients who might be candidates for anti-PSCA antibody therapy. Finally, we wanted to ask whether PSCA expression in bone marrow could identify patients at high risk to recur after initial therapy with prostatectomy.

PROGRESS REPORT

Specific Aim 1: To test the prognostic significance of PSCA overexpression in patients with clinically localized prostate cancer.

Task 1. PSCA in tissue arrays (see appendix). Last year we reported the completion of construction of a prostate cancer tissue microarray linked to a comprehensive patient database. Optimization of staining was completed toward the end of year 1 as well. Over the past year, we have completed the analysis of this array and submitted a manuscript describing our findings. This manuscript has been accepted and is in press in the Journal of Urology. The major results are that PSCA is expressed in virtually all localized tumors and that expression correlates with adverse clinicopathologic features such as seminal vesicle invasion, high Gleason score and extracapsular spread. Patients with the highest level of PSCA expression had a higher risk of PSA relapse following surgery in a univariate analysis. However, PSCA was not an independent predictor of recurrence. Also of note, we obtained the same result when we compared expression in cancer to adjacent normal tissue from the same patient.

Task 2: MYC/PSCA gene amplification: In collaboration with Dr. Robert Jenkins at Mayo clinic, we will perform FISH analysis for MYC and PSCA in order to determine the frequency of gene amplification and the relationship between the two genes. FISH analysis is underway at Mayo, following which we will stain the same arrays for PSCA protein expression.

Task 3: PSCA in biopsies and matched prostatectomies. 50 paired biopsy and prostatectomy specimens have been collected retrospectively. These should be stained over the coming year to determine the relationship of PSCA expression in biopsies and surgical specimens. In addition, we plan a prospective study to determine the association of PSCA expression in biopsies with recurrence.

Specific Aim 2: Prognostic significance of PSCA in blood and bone marrow samples.

We have continued to develop appropriate assay using magnetic bead technology and plan to begin to collect bone marrow biopsies once we have obtained IRB approval from the DOD. The UCLA IRB approved our consent and proposal. A consent form and protocol were submitted to the DOD as well; however, we have yet to receive a response from them.

Nevertheless, we have made progress on Aim 2. First, we obtained a large number of matched metastatic specimens from Dr. Robert Vessella at University of Washington and from Dr. Robert Jenkins at Mayo clinic. These have been stained and are summarized in Table 1 below. PSCA is expressed in a majority of bone metastases. Interestingly, PSCA expression is often higher in bone than in matched soft tissue metastases from the same patient, raising a number of important questions regarding PSCA function and regulation in bone. In addition, in collaboration with Dr. Vessella's group, we have looked at PSCA mRNA expression in bone marrows of patients with metastatic and localized prostate cancer. To date, this experiment has not been successful secondary to false positive expression of PSCA in normal bone marrow. This is in disagreement with our own analyses at UCLA and is being addressed. I believe the test at UW is too sensitive and that fewer PCR cycles will be needed to regain the specificity of PSCA expression for cancer.

Table 1. PSCA expression in metastasis.

	0	1+	2+	3+	Total
Soft Tissue	4(40%)	3(30%)	2(20%)	1(10%)	60%
Bone	7(15%)	7(15%)	13(27%)	20(43%)	85%

KEY RESEARCH ACCOMPLISHMENTS OVER YEAR 2:

1. Completion of PSCA staining, scoring and analysis.
2. Manuscript acceptance
3. Submission of protocol for Aim 2 to DOD
4. PSCA expression analysis in metastatic prostate cancer

REPORTABLE OUTCOMES

Manuscript in Press at Journal of urology entitled "Prostate stem cell antigen expression is associated with Gleason score, seminal vesicle invasion and capsular invasion in prostate cancer"

CONCLUSION

We have made progress towards defining PSCA's prognostic value. These studies identify which patients are likely to have high levels or PSCA expression and who may

be candidates for therapy targeting this antigen. The expression analysis in metastatic cancer has provided biological leads and also validates PSCA as a potential target for therapy. We hope to have preliminary data regarding the utility of PSCA expression in bone marrow aspirates and to begin a prospective trial testing this hypothesis over the coming year.

PSCA expression is associated with Gleason score, seminal vesicle invasion and capsular invasion in prostate cancer. Han KY, Seligson DB, Liu X, Horvath S, Shintaku PI, Thomas GV, Said JW, Reiter RE. In Press JUrology 2003/4

Appendix: PSCA expression is associated with Gleason score, seminal vesicle invasion and capsular invasion in prostate cancer. Han KY, Seligson DB, Liu X, Horvath S, Shintaku PI, Thomas GV, Said JW, Reiter RE. In Press JUrology 2003/4

Prostate Stem Cell Antigen (PSCA) expression is associated with Gleason score, seminal vesicle invasion, and capsular invasion in prostate cancer

Ken-ryu Han*, David B Seligson*, Xueli Liu, Steve Horvath, Peter I Shintaku, George V Thomas, Jonathan W Said, and Robert E Reiter

Departments of Urology (KRH, RER), Pathology and Laboratory Medicine (DBS, PIS, GVT, JWS), Human Genetics and Biostatistics (XL, SH)

UCLA David Geffen School of Medicine, Los Angeles, California

*Both authors contributed equally to this work

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Address correspondence to

Robert E Reiter, MD
UCLA Department of Urology
CHS 66-128
10833 Le Conte Avenue
Los Angeles, CA 90095-1738

Tel: 310-794-7224
Fax: 310-206-5343
rreiter@mednet.ucla.edu

Abstract

Introduction: Few successful therapeutic options exist for men who present with metastatic prostate cancer (CaP) or for the 30% of men who recur. The development and characterization of molecular markers is vital to the development of prognostic and therapeutic modalities in CaP. We investigated the expression and potential clinical utility of prostate stem cell antigen (PSCA) in CaP using tissue microarrays (TMA).

Methods: Immunohistochemical analysis using a PSCA monoclonal antibody was performed on TMA's constructed from paraffin-embedded specimens from patients (N=246) who underwent radical retropubic prostatectomy. PSCA staining was correlated with established prognostic factors such as Gleason score, PSA levels, seminal vesicle invasion; in addition, recurrence-free survival was also analyzed.

Results: High PSCA intensity (PSCA intensity of 3) was associated with adverse prognostic features such as Gleason score 7 and above (P = 0.001), seminal vesicle invasion (P = 0.005), and capsular involvement (P = 0.033). On univariate analysis, tumors with a PSCA intensity of 3 carried an increased risk of PSA recurrence (P = 0.031, hazard ratio = 1.77, 95% CI=1.05 – 2.96). However, after adjusting for the above variables, a PSCA intensity of 3 was no longer an independent predictor of PSA recurrence.

Conclusions: We found that high PSCA intensity is significantly associated with adverse prognostic features such as high Gleason score and extra-organ disease. The results of this study suggest that PSCA is a promising tumor marker for the selection of high-risk patients but additional studies are necessary to assess the utility of PSCA in patient biopsies.

Introduction

According to the American Cancer Society, there will be over 220,000 new cases of prostate cancer (CaP) diagnosed in 2003 in the United States, resulting in an estimated 29,000 deaths. [1] Few successful therapeutic options exist for men who present with metastatic disease or for the 30% of men who recur after local treatment. As a result, the identification, development, and characterization of molecular markers are critical to the development of prognostic and therapeutic modalities in CaP.

Prostate stem cell antigen (PSCA) is a 123 amino acid glycoprotein first identified in the LAPC-4 prostate xenograft model. [2] It is a glycosyl phosphatidylinositol-anchored (GPI) cell-surface protein that bears 30% homology to stem cell antigen type 2.[3, 4] Immunohistochemical (IHC) analysis has shown that PSCA expression is detected in 94% and over-expressed in ~40% of clinically localized CaP. [4] In addition, both Gu[4] and Ross[5] have demonstrated that PSCA expression is found in only a small number of normal tissues beyond the prostate, making it a potentially attractive target for therapy. Elevated PSCA expression was shown to correlate with increased tumor stage and Gleason score in one study, but its association with tumor recurrence has not been investigated.[4]

Because of its cell surface expression in CaP, PSCA continues to be evaluated as a diagnostic and therapeutic target. In this report, tissue microarray (TMA) technology is used to further characterize the clinical utility of PSCA. In particular, PSCA expression is correlated with established clinicopathologic covariates and biochemical recurrence.

Materials and Methods

Patients. The study cohort consisted of 246 patients who underwent radical retropubic prostatectomy between 1984 and 1995. Twenty patients who received neoadjuvant hormones were excluded; 15 patients had tumors that were not informative for PSCA due to lack of tumor representation, and another 14 patients had incomplete clinical data for outcomes analysis, leaving 197 patients available for evaluation. Tissue spots from 245 cases were included in the spot distribution of PSCA staining, and 87% of all tissue spots were informative; one case did not have any informative spots. A retrospective analysis for outcome assessment was based on chart review of clinical, laboratory, and pathologic data. The mean age was 63.6 (46-76) years and the median follow-up was 50 months. The maximum time of follow-up was 62 months. PSA recurrence was defined as any PSA level >0.2 ng/mL and rising.

Tissue array construction

Archival tumor specimens were obtained from the Department of Pathology. All prostate tumors were staged according to the 1997 AJCC staging system.[6] Gleason scores were reviewed and confirmed by two pathologists (DBS and JWS). Prostatectomy specimens were processed using transverse section (5 slices); three slices were submitted; in addition, the seminal vesicles, vas deferens, bladder neck, and apical margins were submitted separately. Pathologists were blinded to the clinical outcomes of the patients.

At least three core tissue biopsies (each 0.6 mm in diameter) were taken from selected morphologically representative regions of each paraffin-embedded prostate tumor and precisely arrayed using a custom-built instrument as previously described.[7]

An additional core biopsy was taken from a morphologically benign-appearing region of the same prostate. Benign tissue included both normal glands and glands consistent with a histological diagnosis of benign prostatic hyperplasia. Sections of 4 μm thickness of each tissue array block were then transferred to glass slides using the paraffin sectioning aid system (adhesive coated slides PSA- CS 4x, adhesive tape, UV lamp; Instrumedics Inc., Hackensack, NJ) to support the cohesion of the 0.6 mm array elements. Quality control was assessed by cross-checking the expected histology and grade at each spot on each PSCA stained slide as well as checking H&E staining after every 50 consecutive sections. All TMA were constructed with Institutional Review Board approval.

Immunohistochemistry

The mouse monoclonal antibody (mAb) 1G8 was derived from BALB/c mice immunized with a glutathione S-transferase-PSCA fusion protein as previously described.[3,4] Immunohistochemical staining was performed with anti-PSCA 1G8 antibody (1:50 dilution) using a peroxidase technique with antigen retrieval using heat treatment.[8]

Semiquantitative assessment of the staining was performed by two pathologists using 2 independent methods. The first method quantified the overall intensity of PSCA protein expression. Staining intensity was based on a 4-point scale from a score of 0 to 3, with a score of 3 being recorded as the highest intensity staining. A score of 0 was given to tissue spots that did not stain. There were an average of 2.9 informative invasive tumor spots per patient. The maximal staining intensity seen on tumor spots within each case was used to represent the case PSCA protein expression for the outcomes analysis.

In addition, expression was also scored according to the intensity of staining of tumor glands when compared to normal glands from the same prostate specimen. The same maximal intensity pooling of benign tissues (morphological normal and BPH) in each case was used for this analysis. Staining in the tumor was scored as being greater than, equal to, or less than that seen in matching normal glands of each case. Staining which was greater in tumor than normal was taken as evidence of PSCA over-expression.

There was > 90% interobserver agreement on intensity scoring between the two pathologists.

Statistical analysis

We dichotomized the staining score into 2 categories: strong staining (PSCA intensity=3) versus low staining (PSCA intensity <3), which lead to intuitive biological results.

Associations between PSCA intensity expression and Gleason score, seminal vesicle invasion, capsular penetration, organ-confinement, and preoperative PSA level were tested using the Pearson chi-square test (table 1). The Kruskal-Wallis test was used to determine PSCA protein expression (figure 2). To relate the censored clinical outcome "time to PSA recurrence" with patient characteristics, we used univariate and multivariate Cox regression models. The proportional hazards assumption was verified using Schoenfeld residuals.[9] We visualized the distributions of recurrence free time with Kaplan Meier plots and used the log rank test to test for differences across groups. All p values were two-sided and a p value < 0.05 was considered significant. All statistical analyses were performed using the freely available statistical software R (http://www.r-project.org/).

Results

Immunohistochemical Staining

Figure 1 demonstrates the definitions of the PSCA intensity scores used in this analysis. While cellular staining of PSCA was quite uniform in most epithelial cells, regions of heterogeneous expression were rarely seen (Figure 1A). Figures 1B, C and D demonstrate representations of weak, moderate and strong PSCA staining, respectively. Specimens that did not stain were given a score of 0.

Overall Distribution of PSCA Expression

Figure 2 demonstrates the distribution of PSCA intensity on the tissue arrays. PSCA staining was analyzed on spots that contained tumor, prostatic intraepithelial neoplasia (PIN), or benign tissue (morphological normal and benign prostatic hyperplasia [BPH] tissue). The majority of the staining is concentrated in the moderate and strong intensities for all histological categories. Twenty-nine percent of Gleason grade 4 spots stained strongly (intensity 3) for PSCA, while 33% of grade 5 spots stained strongly. Grade 1 and 2 spots stained moderately (intensity 2) 55% of the time, while another 27% stained strongly. PIN had the highest overall staining, with all spots staining strongly (32%) or moderately (68%). Finally, 18% of spots containing benign tissue stained strongly and 69% stained moderately. Staining in PIN was statistically higher than normal ($P = 0.028$) or all other tumor grades ($P = 0.016$).

PSCA Intensity in Relation to Prognostic Factors

Table 1 reports the results of cross-tabulating PSCA intensity with established prognostic factors in prostate cancer. Forty-five percent of Gleason 7 and above cancers

stained with a PSCA intensity of 3 compared to only 22% of tumors that were Gleason 6 or less (P = 0.001). When the PSCA intensity of tumors with seminal vesicle (SV) invasion was analyzed, 53% of tumors with SV invasion stained with a score of 3, compared to only 26% for tumors with no SV involvement (P = 0.005).

Organ-confined tumors (T2 or less and N0M0) stained with a score of 3 only 24% of the time, compared to 44% for tumors with extra-prostatic involvement (T3a – T4b or nodal disease)(P = 0.010). Finally, 41% of tumors with extra-prostatic extension stained with a score of 3, while only 16% of tumors that did not involve the capsule stained intensely for PSCA (P = 0.033). PSCA staining intensity did not statistically correlate with PSA values using a cut-off of 10ng/mL or with tumors that had associated nodal involvement. These results demonstrate a correlation between PSCA expression and adverse pathological parameters when the tumor alone is scored.

Univariate and Multivariate Cox Regression Analyses

Table 2a demonstrates the results of univariate analysis performed for established prognostic factors for time to PSA recurrence. We found that SV invasion (P< 0.001), Gleason score ≥ 7(P< 0.001), extraprostatic extension (P= 0.003), and a preoperative PSA level > 10ng/mL (P=0 .007) increased the risk of PSA recurrence. Importantly, tumors that stained with maximum PSCA intensity of 3 had an increased hazard ratio of 1.77 (95% CI=1.05 – 2.96, log rank P= 0.031) when compared to tumors with a lower PSCA staining intensity.

Using Kaplan-Meier estimates (Figure 3), we found that patients whose tumor stained with a PSCA intensity of 3 had only a 56% chance of remaining free from PSA recurrence at 5-year follow-up (N = 61). By comparison, a patient with a tumor that

stained with a score of 1 or 2 (N = 136) had a 76% chance of being free from recurrence at 5-year follow-up (P= 0.03)

Table 2b demonstrates the results of the multivariate analysis. We found that only SV invasion (P< 0.001), Gleason score 7 or greater (P= 0.009), and capsular invasion (P= 0.004) remained independent significant predictors of PSA recurrence. PSCA intensity was no longer a significant predictor of PSA recurrence in the multivariate model (Hazard ratio = 0.88, P =0.7).

Normal versus Tumor

Table 3 compares maximal PSCA expression in tumors to that in matched morphologically benign tissue from the same patient. The trend demonstrated by the table indicates that as the Gleason score increased, the proportion of tumor spots with maximal intensity greater than its matched normal also increased. For instance, in Gleason 7 tumors, the tumor stained more intensely 33% of the time; Gleason 8-10 tumors stained more intensely 38% of the time when compared to their matched normal counterparts. By comparison, only 20% of the Gleason 6 or less tumors stained more intensely than their matched normals (P = 0.008).

Discussion

The major finding of this paper, that PSCA expression correlates significantly with Gleason score and tumor stage, is in agreement with a prior study by Gu and associates.[4] As in the present study, Gu also showed that a comparison of PSCA expression in tumor and adjacent normal yielded results similar to an analysis of tumor expression alone. The current study goes beyond the previous study by showing that PSCA overexpression also correlates with an increased risk of biochemical recurrence.

Since only a small number of men in the present study developed a clinical recurrence, we could not assess whether PSCA expression predicts clinical recurrence, metastasis or death. Hara et al. recently reported on the outcomes of men who had circulating PSCA-positive cells at the time of prostate cancer diagnosis. Hara found that patients with detectable PSCA-positive circulating cells had a higher mean Gleason score (5.71) than those who were PSCA negative (4.14) ($P < 0.05$). PSCA positivity also correlated with extra-prostatic extension. Whereas all patients with organ-confined disease were PSCA negative, 47% of patients with extra-organ disease were PSCA positive.[10] Most importantly, patients that were PSCA PCR (+) had a significantly worse disease-specific survival when compared to their counterparts that were PSCA PCR (-). Additional studies of PSCA expression in tumors that progress clinically and in micrometastases will be necessary to draw conclusions regarding the prognostic significance of PSCA expression.

The current findings have a number of potential clinical implications. First, the association of PSCA expression with tumor stage raises the possibility that PSCA intensity on biopsy specimens could potentially be used in a predictive manner. For example, a positive biopsy that also stains with a PSCA intensity of 3 might be used to counsel a patient that he may have an increased risk of seminal vesicle invasion or extra-organ disease. This information could eventually be incorporated into nomograms similar that are similar to the Partin nomogram.[11] We are currently addressing the question of PSCA expression in prospective patient biopsies.

Perhaps the most important potential application of the current results relates to therapy. We and others have shown that monoclonal antibodies targeting PSCA can

inhibit tumor growth and prevent metastasis in mouse models of human prostate cancer.[3,5] A humanized version of PSCA monoclonal antibody 1G8 has now been generated (unpublished data) and may be ready for Phase I studies in the near future.

The expression profile of PSCA reported here supports PSCA as a therapeutic target and may identify a subgroup of patients who best qualify for therapy (i.e. patients whose primary tumors express high levels of PSCA). One promising clinical application of monoclonal therapy is as an adjuvant. Because PSCA expression intensity correlates with increased risk of recurrence, postoperative patients with high-risk parameters and high PSCA expression (or PSCA positive micrometastases defined by RT-PCR) could be included in clinical studies of adjuvant PSCA antibody.

Although the results of the current study are in agreement with a number of previous studies, it is noteworthy that Ross and associates did not confirm a positive correlation between the level of PSCA mRNA expression and high Gleason grade.[5] One possible explanation is that Ross et al. used *in situ* hybridization (ISH) technology to measure PSCA mRNA levels, while the current study measures PSCA protein expression. PSCA may be in part regulated post-transcriptionally, potentially explaining the overall difference in positivity as well as the lack of correlation with tumor grade. A second possible explanation is that the Ross study grouped tumors into Gleason </= 7 and Gleason > 7, whereas we classified tumors into Gleason < 7, Gleason = 7, and Gleason >7 (table 3). Any correlation of PSCA with tumor grade could have been lost by this difference in stratification, since Gleason 7 tumors in our study were more likely than Gleason 6 or less tumors to express higher levels of PSCA. In agreement with Ross, we did not find any overall difference in PSCA positivity between normal prostate and

prostate cancer. However, unlike the current study, Ross and colleagues did not compare PSCA expression in matched tumor and morphologically normal samples.

The functional role of PSCA and the mechanisms governing elevated PSCA expression in prostate carcinogenesis remain poorly understood. The PSCA gene is located on chromosome 8q24.2, distal to the *MYC* oncogene. Amplification of MYC has been identified in a significant percentage of primary and metastatic prostate cancers.[12] Reiter et al. showed that PSCA overexpression might be attributed to PSCA and MYC amplification in some locally advanced tumors.[13]

A number of investigators have shown that the murine homologue of PSCA is upregulated in transgenic models of prostate cancer and PIN, analogous to the overexpression of human PSCA reported here. For example, PSCA is overexpressed in TRAMP (transgenic adenocarcinoma mouse prostate), PTEN heterozygous, and NKX 3.1 knockout mice.[14-17] Similarly, Watabe and colleagues crossed the TRAMP mouse with a transgenic mouse model in which the PSCA promoter was used to drive expression of enhanced green fluorescent protein (GFP). GFP expression in the resulting cancers was much more intense and pervasive than in wild type control mice. These results suggest that PSCA overexpression in cancer may also be regulated at the transcriptional level.[18]

Finally, Tran and associates reported that PSCA is a marker of transit-amplifying prostate epithelial cells in tissue culture. [19] These investigators speculated that PSCA overexpression in cancer might be caused by an expansion of a transformed PSCA-positive cell. A similar phenomenon could explain the overexpression of PSCA in the above-mentioned transgenic models of prostate cancer.

In conclusion, increased expression of PSCA is associated with adverse prognostic factors such as high Gleason score and extra-organ disease. PSCA overexpression correlates with an increased risk of biochemical recurrence. Although additional work will be needed to assess the utility of PSCA in patient biopsies, our results support the use of PSCA as a marker guiding the selection of high-risk patients for clinical trials of PSCA immunotherapy.

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Table 1. Associating PSCA intensity with clinical prognostic factors. There were 197 patients available for evaluation.

Variable				**P value***
		PSCA intensity 1 or 2	**PSCA intensity 3**	
Gleason score	≤ 6	93/119 (78%)	26/119 (22%)	**0.001**
	≥ 7	43/ 78 (55%)	35/78 (45%)	
		PSCA intensity 1 or 2	**PSCA intensity 3**	
Seminal Vesicle (SV) Invasion	No invasion	120/163 (74%)	43/163 (26%)	**0.005**
	SV invasion	16/34 (47%)	18/ 34 (53%)	
		PSCA intensity 1 or 2	**PSCA intensity 3**	
Organ-confined	Yes	97/128 (76%)	31/128 (24%)	**0.010**
	No	38/67 (56%)	29/67 (44%)	
		PSCA intensity 1 or 2	**PSCA intensity 3**	
Capsular Involvement	None	37/44 (84%)	7/44 (16%)	**0.033**
	Extra-prostatic extension	76/114 (67%)	38/114 (33%)	
	extra-capsular extension	23/39 (59%)	16/39 (41%)	
		PSCA intensity 1 or 2	**PSCA intensity 3**	
PSA value	< 10 ng/mL	69/95 (73%)	26/95 (27%)	0.21
	≥ 10 ng/mL	48/80 (60%)	32/80 (40%)	
		PSCA intensity 1 or 2	**PSCA intensity 3**	
Nodal involvement	No	129/184 (70%)	55/184 (30%)	0.45
	Yes	6/11 (55%)	5/11 (45%)	

*P values = Pearson chi-square test

Table 2A. Univariate Cox regression analysis

Variable	Relative risk	95% CI	P value
SV invasion	4.52	2.67 –7.63	< 0.001
Gleason ≥ 7	4.06	2.38 –6.94	< 0.001
Extra-prostatic extension	2.22	1.30 – 3.78	0.003
pre-op PSA ≥ 10	2.16	1.24 –3.76	0.007
PSCA intensity = 3	1.77	1.05 – 2.96	0.031

Table 2B. Multivariate Cox regression analysis

Variable	Relative risk	95% CI	P value
SV invasion	3.22	1.66 – 6.25	< 0.001
Gleason ≥ 7	2.58	1.27 – 5.24	0.009
Extra-prostatic extension	2.35	1.31 – 4.22	0.004
pre-op PSA ≥ 10	1.32	0.70 – 2.43	0.37
PSCA intensity = 3	0.88	0.47 – 1.66	0.7

Table 3. Tumor versus Normal

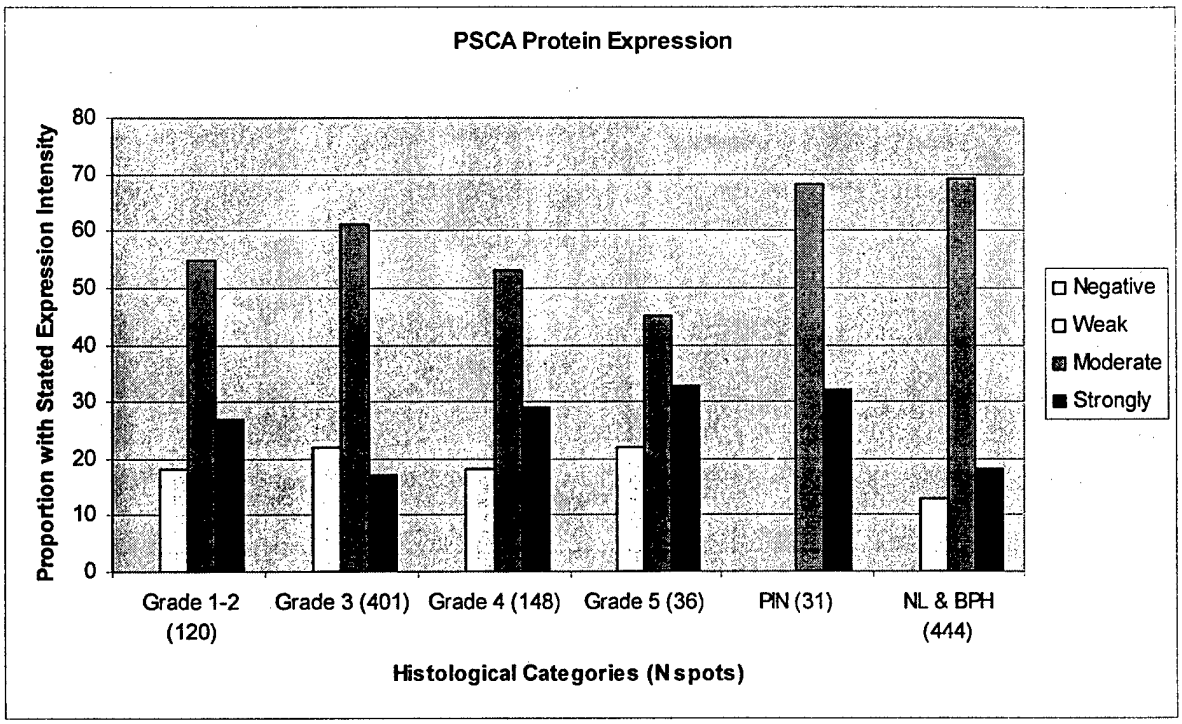
PSCA Tumor vs. normal (P = 0.008)				
Gleason Score	**T<N (%)**	**T=N (%)**	**T>N (%)**	**Unknown (NA)**
2 to 6	32 (26%)	68 (54%)	25 (20%)	20
7	9 (14%)	35 (53%)	22 (33%)	13
8 -10	5 (31%)	5 (31%)	6 (38%)	6
Overall	46 (22%)	108 (52%)	53 (26%)	39

P value = Pearson chi-square test

Figures and Legends

Figure 1. (A) An example of heterogeneous staining with PSCA antibody (rare case)
(B) Weak stain or PSCA intensity = 1
(C) Moderate stain or PSCA intensity = 2
(D) Strong stain or PSCA intensity = 3
Whole spot images at 10x and inserts at 40x.

Figure 2. Distribution graph. The numbers in parentheses indicate the number of spots available for evaluation. There were a total of 1180 spots available for analysis.



Negative	= PSCA intensity 0
Weak	= PSCA intensity 1
Moderate	= PSCA intensity 2
Strongly	= PSCA intensity 3
PIN	= Prostatic intraepithelial neoplasia
NL	= Normal tissue
BPH	= Benign prostatic hyperplasia

PIN (31) shows significantly higher staining than NL & BPH (444) (P value = 0.028, Kruskal-Wallis test)
PIN (31) shows significantly higher expression than all Gleason grades combined (P value = 0.016, Kruskal-Wallis test)

Figure 3. Kaplan-Meier plot comparing the likelihood of remaining free from PSA recurrence (PSA > 0.2 ng/mL) depending on PSCA intensity (P = 0.03).